FORENSIC DRUG REVIEW

Differentiation of AB-FUBINACA Isomers

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Table of contents:

Part 1: USACIL Data:

Compound Information
Figure 1. AB-FUBINACA GC/MS
Figure 2. Isomer 1 GC/MS
Figure 3. Isomer 2 GC/MS
Figure 4. Isomer 3 GC/MS
Figure 5. Isomer 4 GC/MS
Figure 6. Isomer 5 GC/MS
Figure 7. Isomer 6 Cyclized Degradant GC/MS
Figure 8. Peak 1 Isomer 6 Mass Spectrum
Figure 9. Peak 2 Isomer 6 Cyclized Degradant Mass Spectrum
Figure 10. GC/MS overlay 1
Figure 11. GC/MS overlay 2
Figure 12. AB-FUBINACA GC/IR
Figure 13. Isomer 1 GC/IR
Figure 14. Isomer 2 GC/IR
Figure 15. Isomer 3 GC/IR
Figure 16. Isomer 4 GC/IR
Figure 17. Isomer 5 GC/IR
Figure 18. Isomer 6 Cyclized Degradant GC/IR
Figure 19. GC-IR Expanded Fingerprint Region Comparison

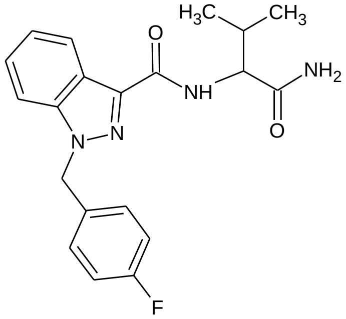
Part 2: External Links:

SWGDRUG Monographs

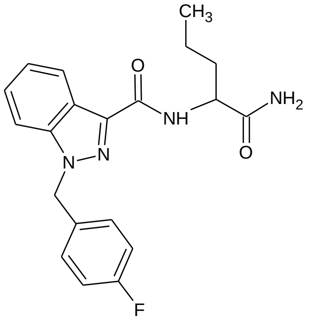
Forendex

Part 1. USACIL Data

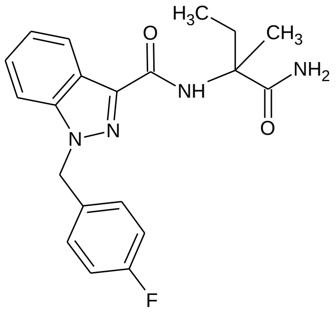
Compound Information



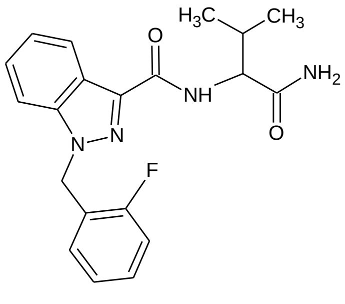
Name: AB-FUBINACA
Synonyms: *N*-(1-amino-3-methyl-1-oxobutan-2-yl)-1-(4-fluorobenzyl)-1*H*-indazole-3-carboxamide
CAS#: 1185282-01-2
MF: C20H21FN4O2
MW: 368.4047 g/mol
SMILES: CC(C(C(=O)N)NC(=O)c1nn(c2c1cccc2)Cc1ccc(cc1)F)C
InChl Key: AKOOIMKXADOPDA-UHFFFAOYSA-N
InChl: InChl=1S/C20H21FN4O2/c1-12(2)17(19(22)26)23-20(27)18-15-5-3-4-6-16(15)25(24-18)11-13-7-9-14(21)10-8-13/h3-10,12,17H,11H2,1-2H3,(H2,22,26)(H,23,27)



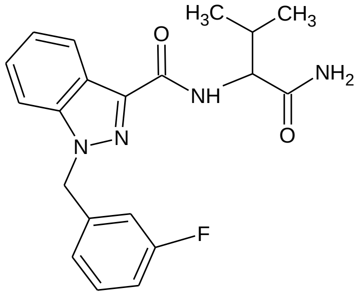
Name: Isomer 1
Synonyms: *N*-(1-amino-1-oxopentan-2-yl)-1-(4-fluorobenzyl)-1*H*-indazole-3-carboxamide
CAS#: N/A
MF: C20H21FN4O2
MW: 368.4047 g/mol
SMILES: NC(C(CCC)NC(=O)C1=NN(C2=CC=CC=C12)CC1=CC=C(C=C1)F)=O
InChl Key: VDGIZMNHDDBEBU-UHFFFAOYSA-N
InChl: InChl=1/C20H21FN4O2/c1-2-5-16(19(22)26)23-20(27)18-15-6-3-4-7-17(15)25(24-18)12-13-8-10-14(21)11-9-13/h3-4,6-11,16H,2,5,12H2,1H3,(H2,22,26)(H,23,27)/f/h23H,22H2



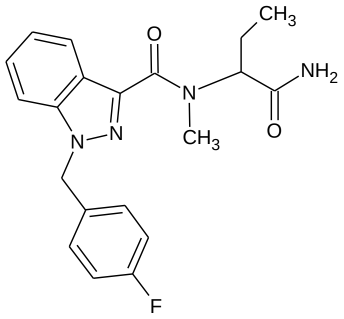
Name: Isomer 2
Synonyms: N-(1-amino-2-methyl-1-oxobutan-2-yl)-1-(4-fluorobenzyl)-1H-indazole-3-carboxamide
CAS#: N/A
MF: C20H21FN4O2
MW: 368.4047 g/mol
SMILES: NC(C(CC)(C)NC(=O)C1=NN(C2=CC=CC=C12)CC1=CC=C(C=C1)F)=O
InChl Key: SGXRGVNSKNQWSC-UHFFFAOYSA-N
InChl: InChl=1/C20H21FN4O2/c1-3-20(2,19(22)27)23-18(26)17-15-6-4-5-7-16(15)25(24-17)12-13-8-10-14(21)11-9-13/h4-11H,3,12H2,1-2H3,(H2,22,27)(H,23,26)/f/h23H,22H2



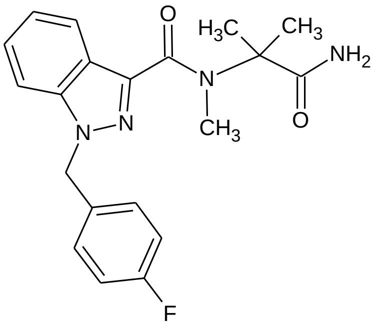
Name: Isomer 3
Synonyms: *N*-(1-amino-3-methyl-1-oxobutan-2-yl)-1-(2-fluorobenzyl)-1*H*-indazole-3-carboxamide
CAS#: N/A
MF: C20H21FN4O2
MW: 368.4047 g/mol
SMILES: NC(C(C(C)C)NC(=O)C1=NN(C2=CC=CC=C12)CC1=C(C=CC=C1)F)=O
InChl Key: AJGUHANHCUPXSK-UHFFFAOYSA-N
InChl: InChl=1/C20H21FN4O2/c1-12(2)17(19(22)26)23-20(27)18-14-8-4-6-10-16(14)25(24-18)11-13-7-3-5-9-15(13)21/h3-10,12,17H,11H2,1-2H3,(H2,22,26)(H,23,27)/f/h23H,22H2



Name: Isomer 4
Synonyms: N-(1-amino-3-methyl-1-oxobutan-2-yl)-1-(3-fluorobenzyl)-1H-indazole-3-carboxamide
CAS#: N/A
MF: C20H21FN4O2
MW: 368.4047 g/mol
SMILES: NC(C(C(C)C)NC(=O)C1=NN(C2=CC=CC=C12)CC1=CC(=CC=C1)F)=O
InChl Key: PDYYWSOYXNOKBK-UHFFFAOYSA-N
InChl: InChI=1/C20H21FN4O2/c1-12(2)17(19(22)26)23-20(27)18-15-8-3-4-9-16(15)25(24-18)11-13-6-5-7-14(21)10-13/h3-10,12,17H,11H2,1-2H3,(H2,22,26)(H,23,27)/f/h23H,22H2



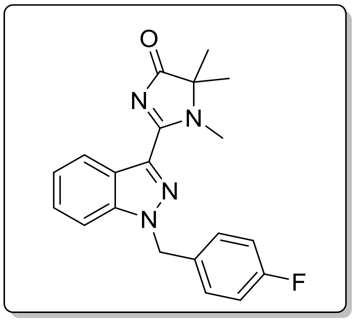
Name: Isomer 5
Synonyms: *N*-(1-amino-1-oxobutan-2-yl)-1-(4-fluorobenzyl)-*N*-methyl-1*H*-indazole-3-carboxamide
CAS#: N/A
MF: C20H21FN4O2
MW: 368.4047 g/mol
SMILES: NC(C(CC)N(C(=O)C1=NN(C2=CC=CC=C12)CC1=CC=C(C=C1)F)C)=O
InChl Key: WEURZBLJVAHFOS-UHFFFAOYSA-N
InChl: InChI=1/C20H21FN4O2/c1-3-16(19(22)26)24(2)20(27)18-15-6-4-5-7-17(15)25(23-18)12-13-8-10-14(21)11-9-13/h4-11,16H,3,12H2,1-2H3,(H2,22,26)/f/h22H2



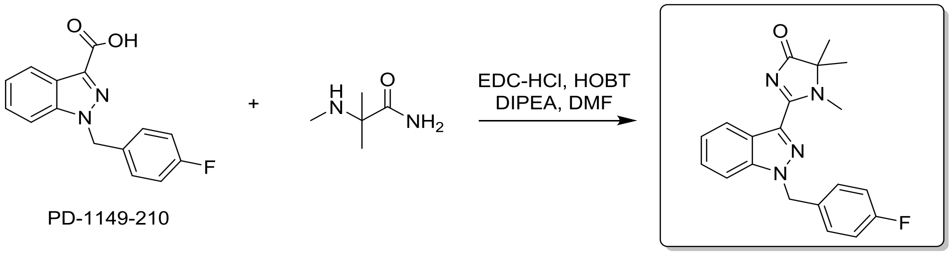
Name: Isomer 6
Synonyms: *N*-(1-amino-2-methyl-1-oxopropan-2-yl)-1-(4-fluorobenzyl)-*N*-methyl-1*H*-indazole-3-carboxamide
CAS#: N/A
MF: C20H21FN4O2
MW: 368.4047 g/mol
SMILES: NC(C(C)(C)N(C(=O)C1=NN(C2=CC=CC=C12)CC1=CC=C(C=C1)F)C)=O
InChl Key: PRZLRKHYGFTMRL-UHFFFAOYSA-N
InChl: InChI=1/C20H21FN4O2/c1-20(2,19(22)27)24(3)18(26)17-15-6-4-5-7-16(15)25(23-17)12-13-8-10-14(21)11-9-13/h4-11H,12H2,1-3H3,(H2,22,27)/f/h22H2

Background of AB-FUBINACA Isomer 6 (Provided by Cayman Chemical Company):

In the attempted synthesis of AB-FUBINACA Isomer 6, it was discovered that upon normal amide coupling conditions (N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC), 1-Hydroxybenzotriazole (HOBT), and N,N-Diisopropylethylamine (DIPEA) using Dimethylformamide (DMF) as the reaction solvent) the major product of the reaction was a cyclized degradant shown below.

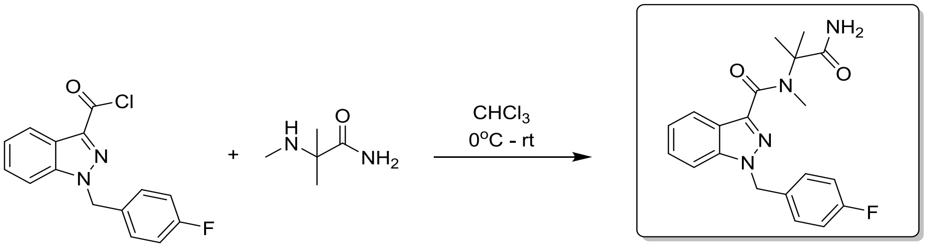


Intramolecular cyclization has been shown to occur in similar type compounds under basic conditions (US 2010/0113546 A1). A purified sample of the cyclized product was provided along with LC/MS and ^{1}H NMR results. To form the desired isomer it was discovered that the reaction needed to proceed using the acid chloride in presence of the amine with no base in the reaction. A pure sample of Isomer 6 was unable to be obtained. However, a sample of 13mg of 86% pure material was obtained with the only impurity being hydrolyzed starting material (acid chloride) to the acid (PD-1149-210). As can be seen in figure 7, the majority of the compound apparently converts to the cyclized form of isomer 6 in the injection port of the gas chromatograph.



PD-1149-210

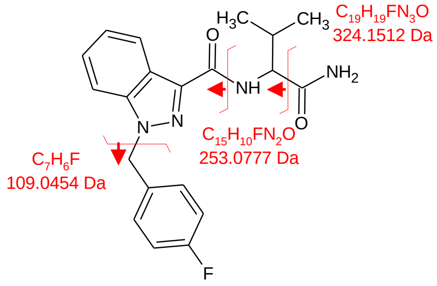
Chemical Formula: $C_{20}H_{19}FN_4O$
Molecular Weight: 350.40
AB-Fubinaca Cyclized Isomer 6



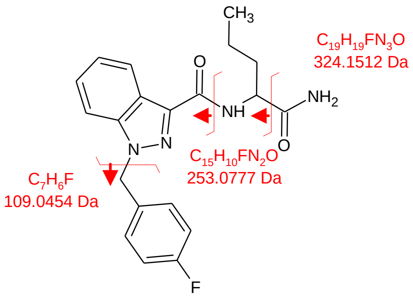
Chemical Formula: $C_{20}H_{21}FN_4O_2$
Molecular Weight: 368.41
AB-Fubinaca Isomer 6
13mg of 86% purity
14% impurity of PD-1149-210

Six isomers of AB-FUBINACA which were predicted to have similar mass spectra to that of AB-FUBINACA were synthesized. The major ions formed in all of these isomers were m/z=324, m/z= 253, and m/z= 109.

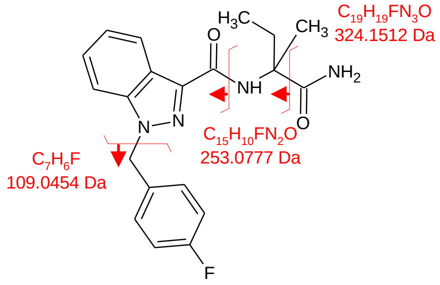
AB-FUBINACA



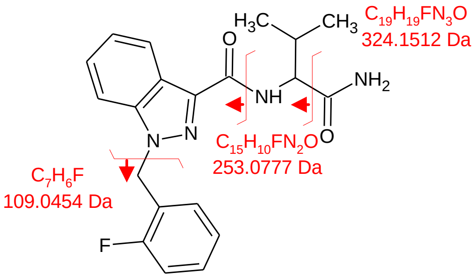
Isomer 1



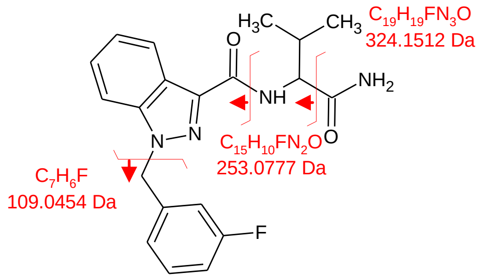
Isomer 2



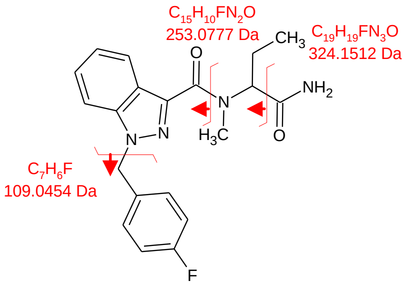
Isomer 3



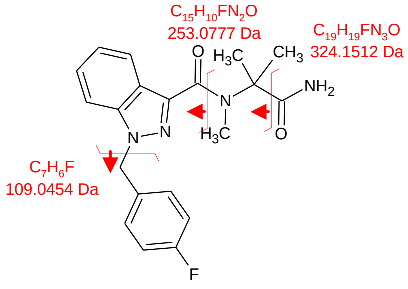
Isomer 4



Isomer 5



Isomer 6



Gas chromatography / Mass spectrometry

Sample Preparation: Approximately 1 mg/mL in methanol

Instrument: Agilent 7890A GC/ 5975C MS

GC Parameter: **Column:** HP-5MS 30m× 250µm× .25µm

Carrier Gas: He

Oven Program: 240°C for 1 minute, ramp 30°C/minute to 300°C and hold for 11 minutes.

Injection parameter: **Injector Temp:** 250°C

MS Parameters: **MSD transfer line:** 290°C

MS Source: 230°C

MS Quad: 150°C

Mass Scan Range: 34-550 amu

Threshold: 150

Tune File: atune.u

Compound	Retention Time	Relative Retention Time
AB-FUBINACA	5.080	1.000
Isomer 1	5.365	1.056
Isomer 2	4.951	.975
Isomer 3	4.976	.980
Isomer 4	5.037	.992
Isomer 5	4.876	.960
Isomer 6A (Isomer 6 cyclized analog)	5.772	1.136

Figure 1. AB-FUBINACA GC-MS

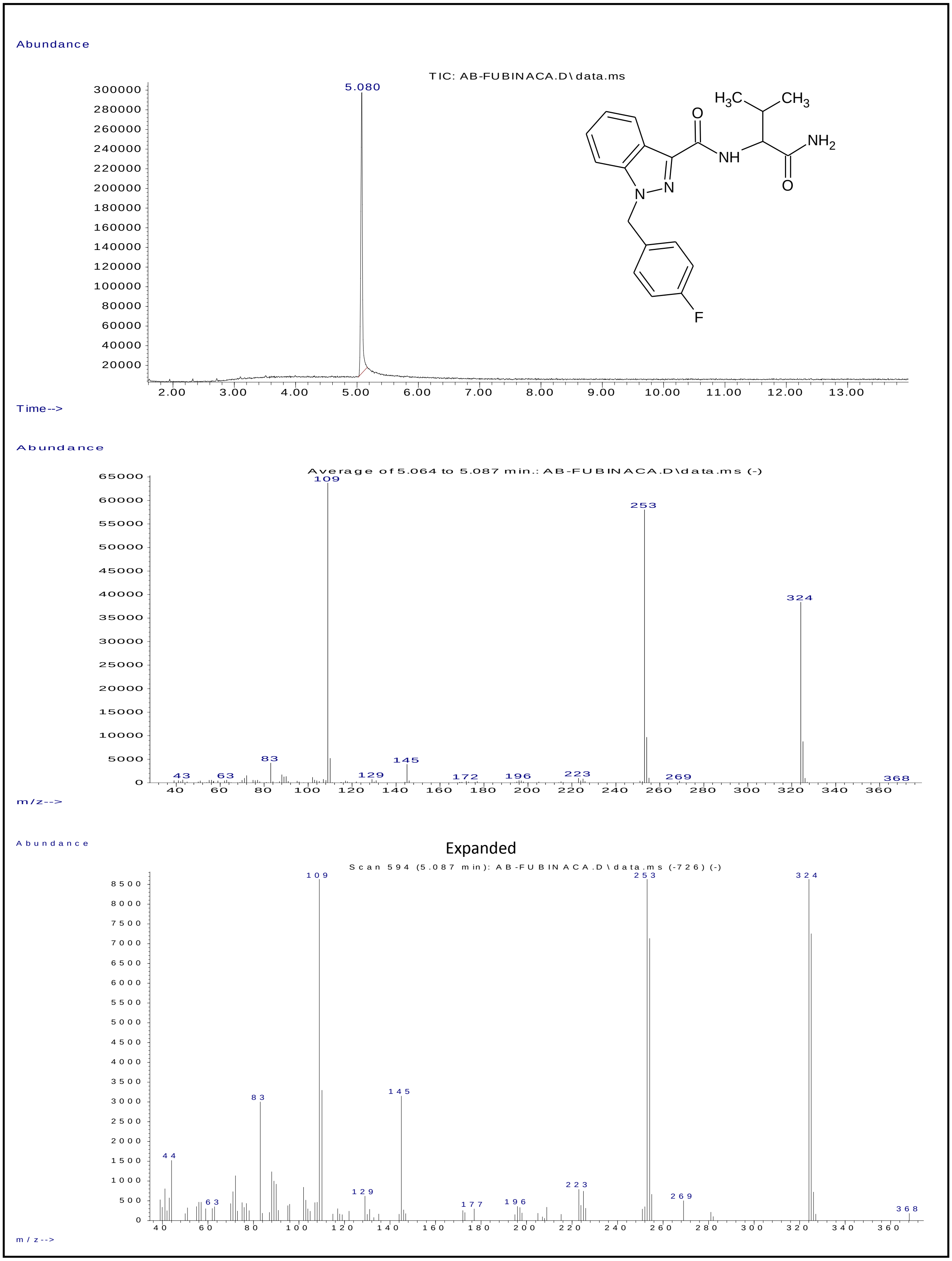


Figure 2. Isomer 1 GC-MS

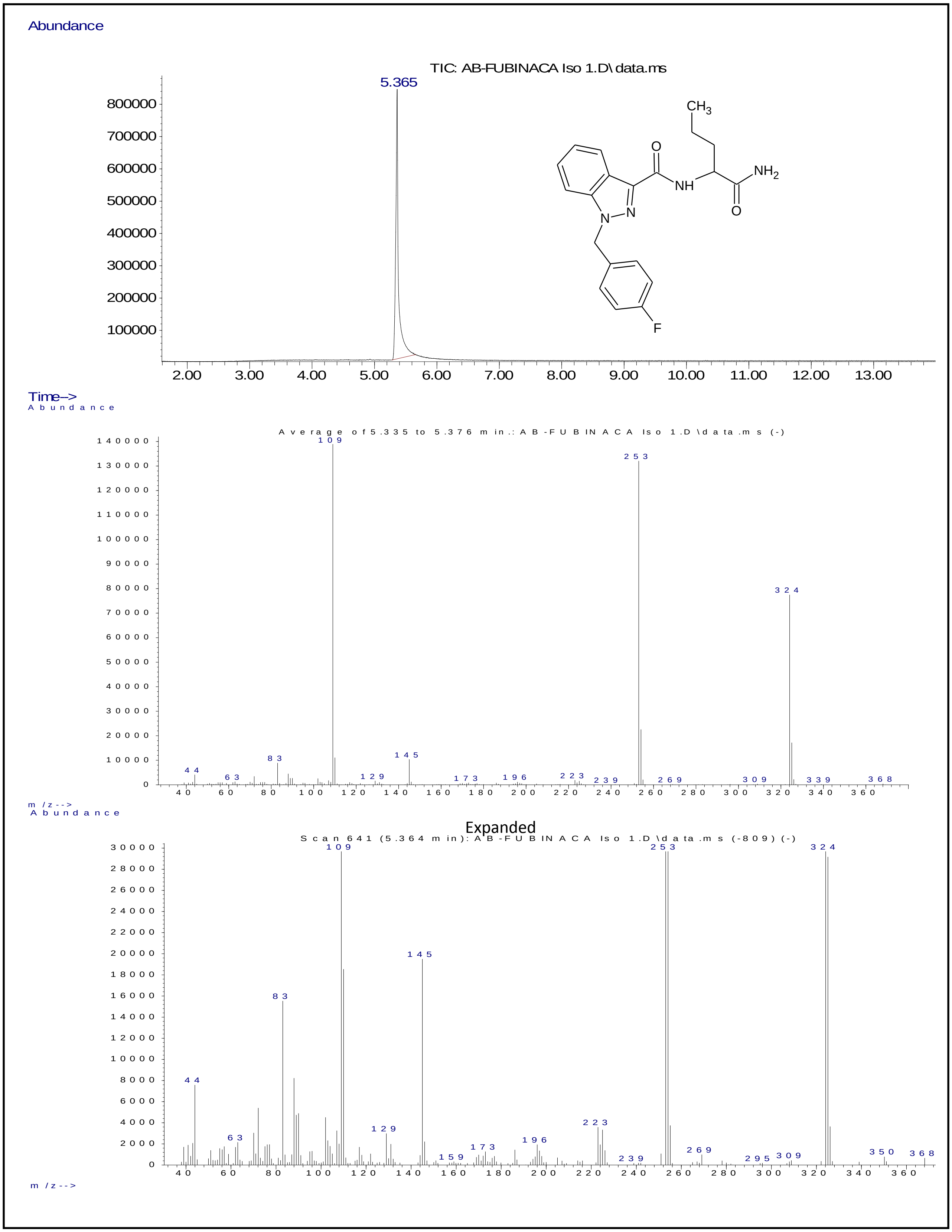


Figure 3. Isomer 2 GC-MS

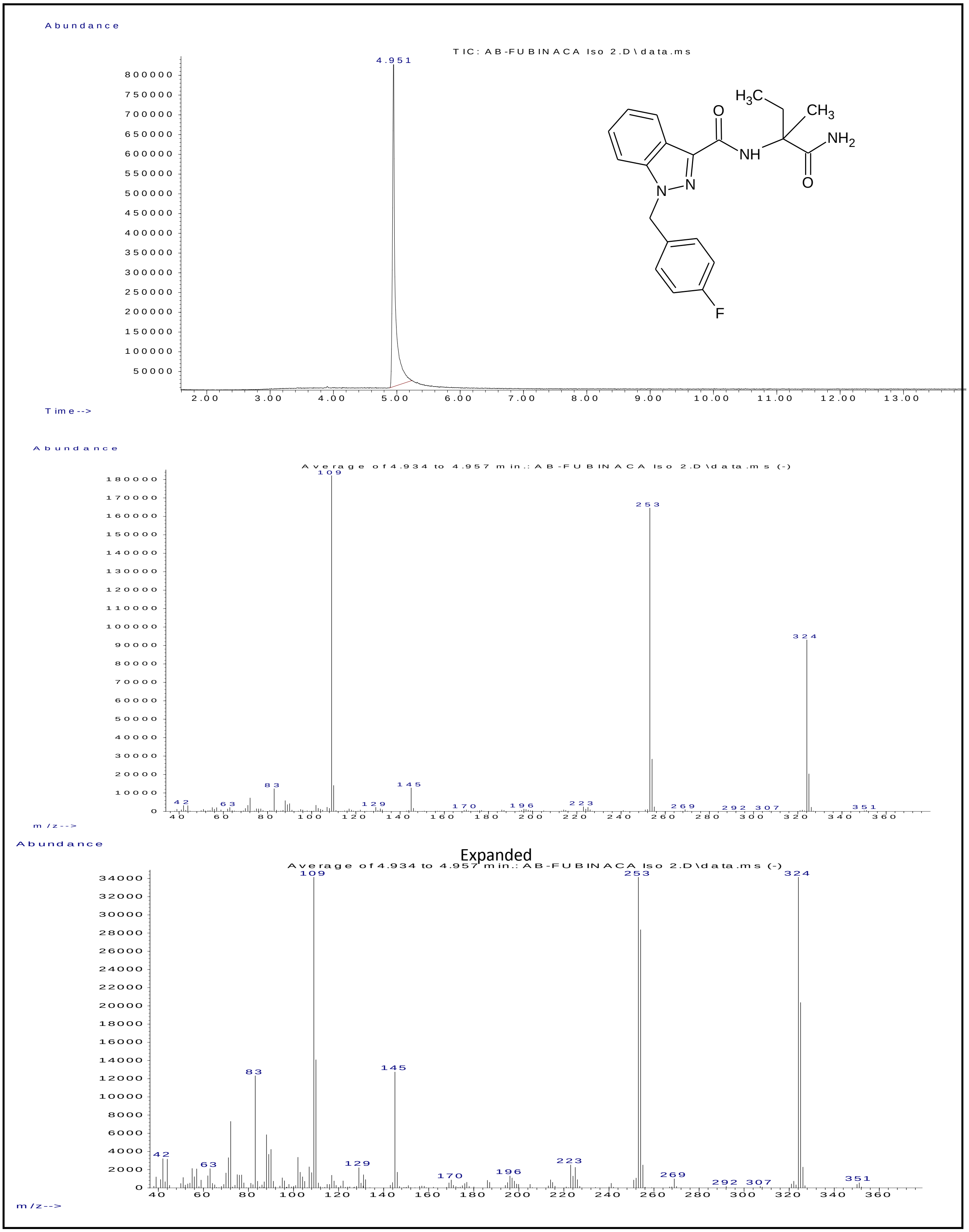


Figure 4. Isomer 3 GC-MS

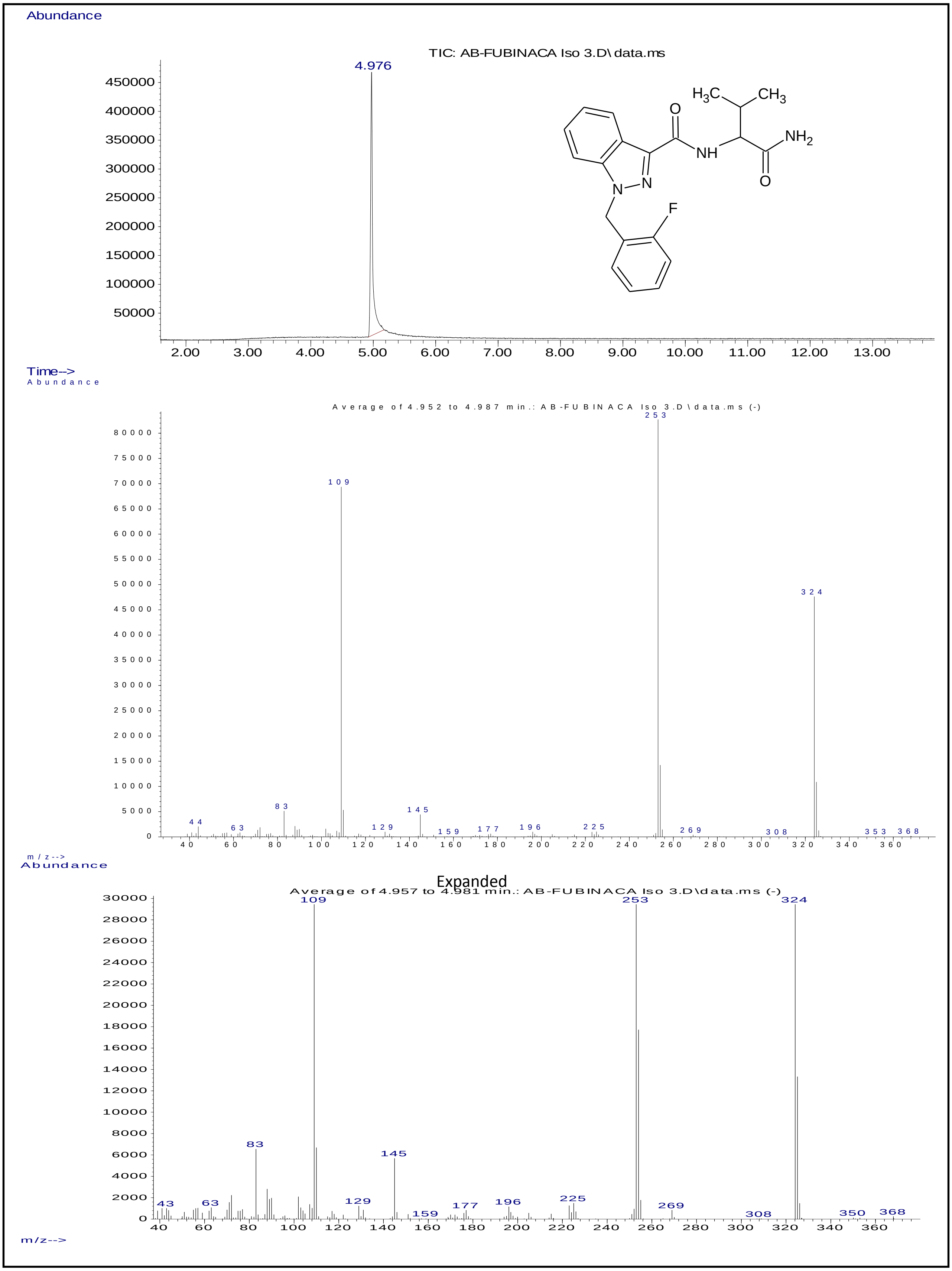


Figure 5. Isomer 4 GC-MS

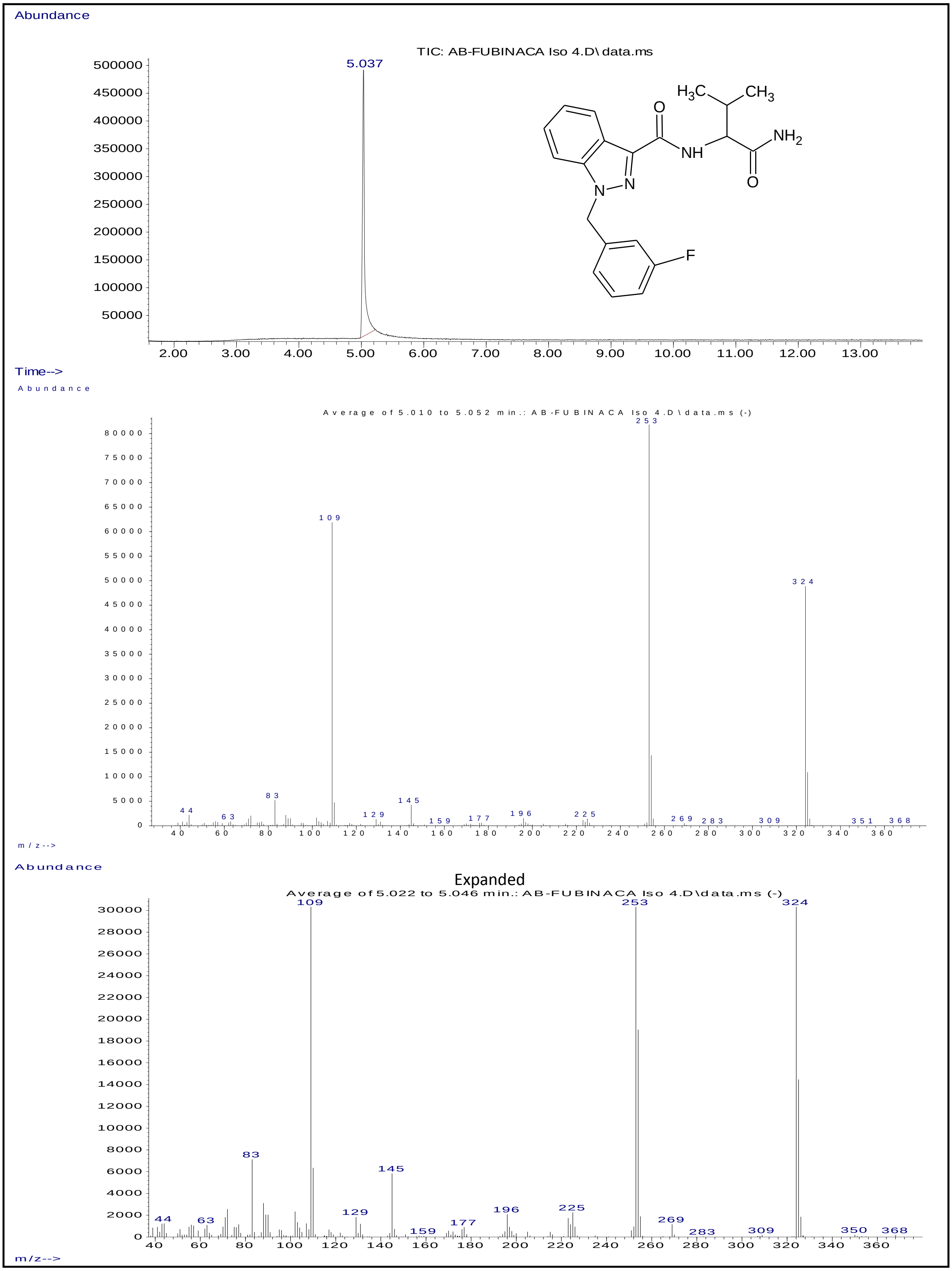
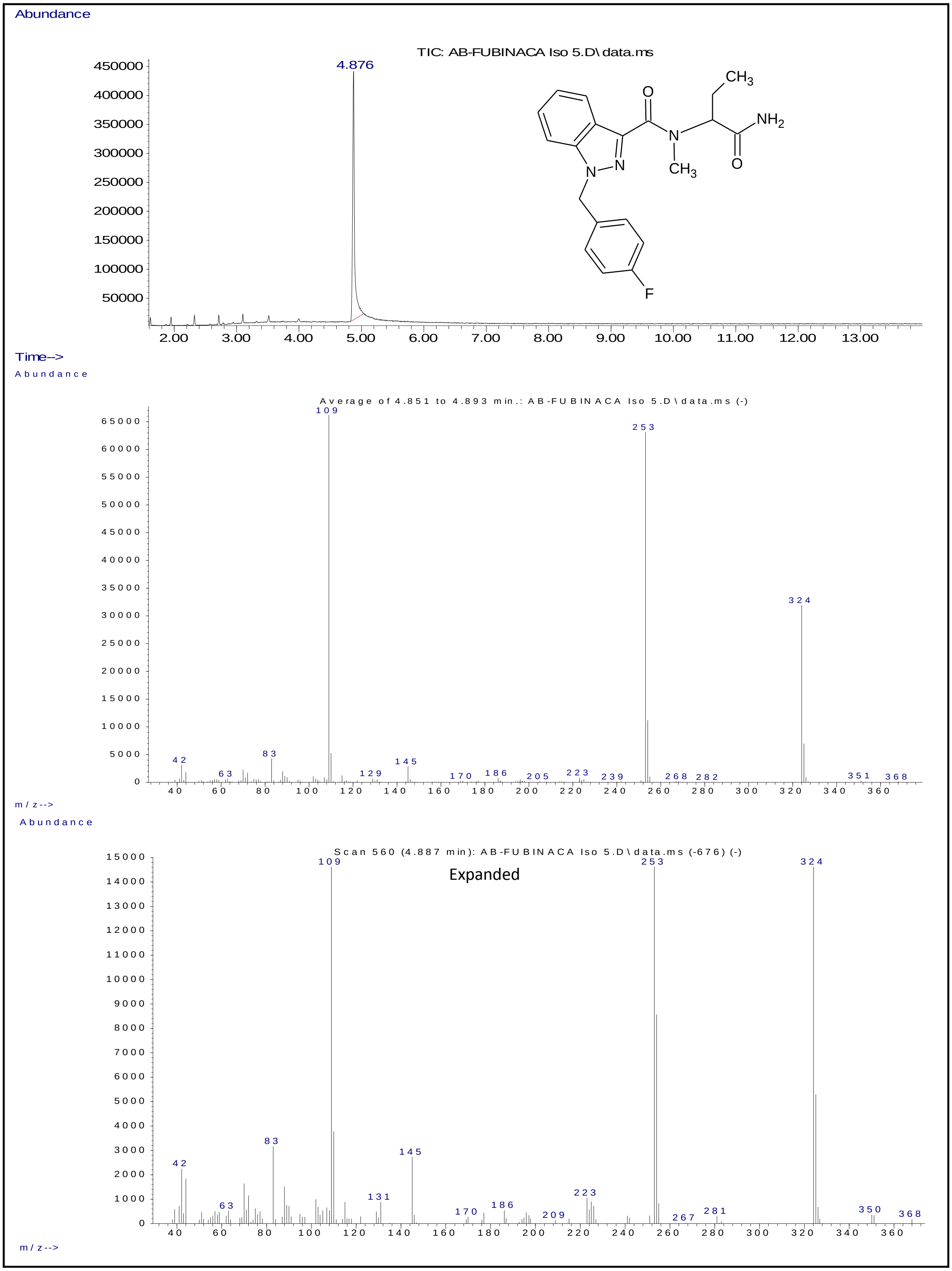


Figure 6. Isomer 5 GC-MS



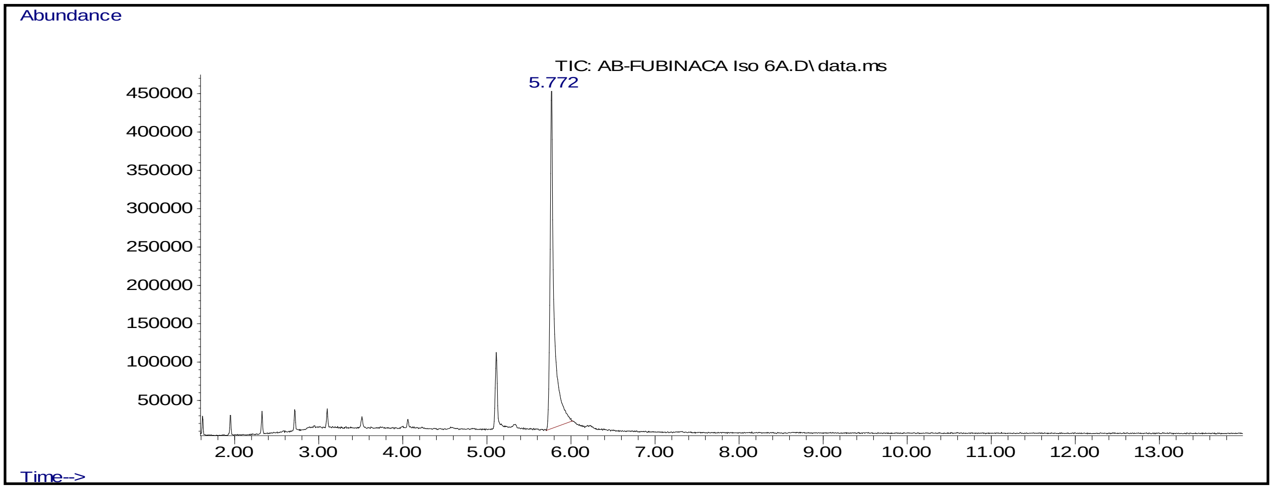


Figure 7. Isomer 6 Cyclized Degradant GC-MS

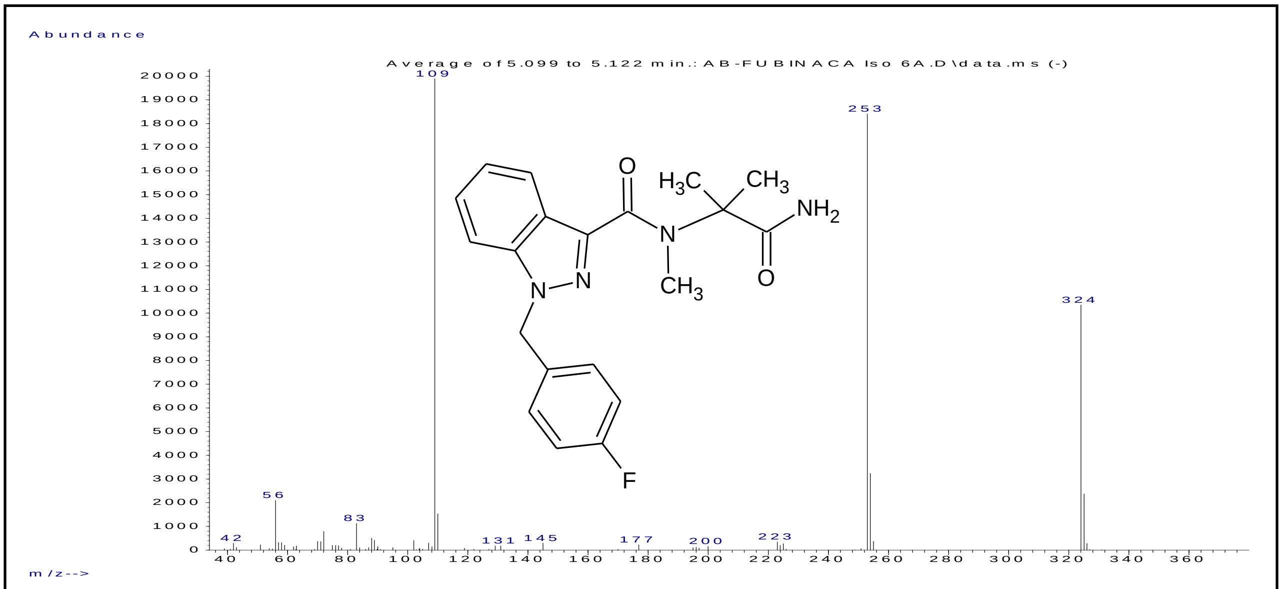


Figure 8. Peak 1 Mass Spectrum: Isomer 6

Expanded

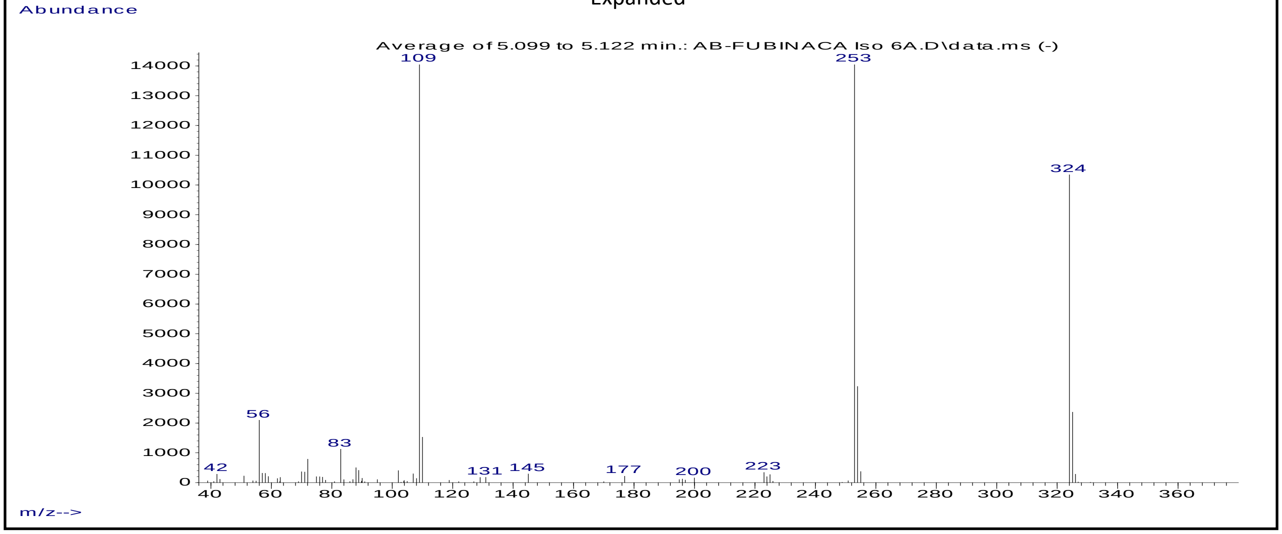


Figure 9. Peak 2 Mass Spectrum: Isomer 6 Cyclized Degradant

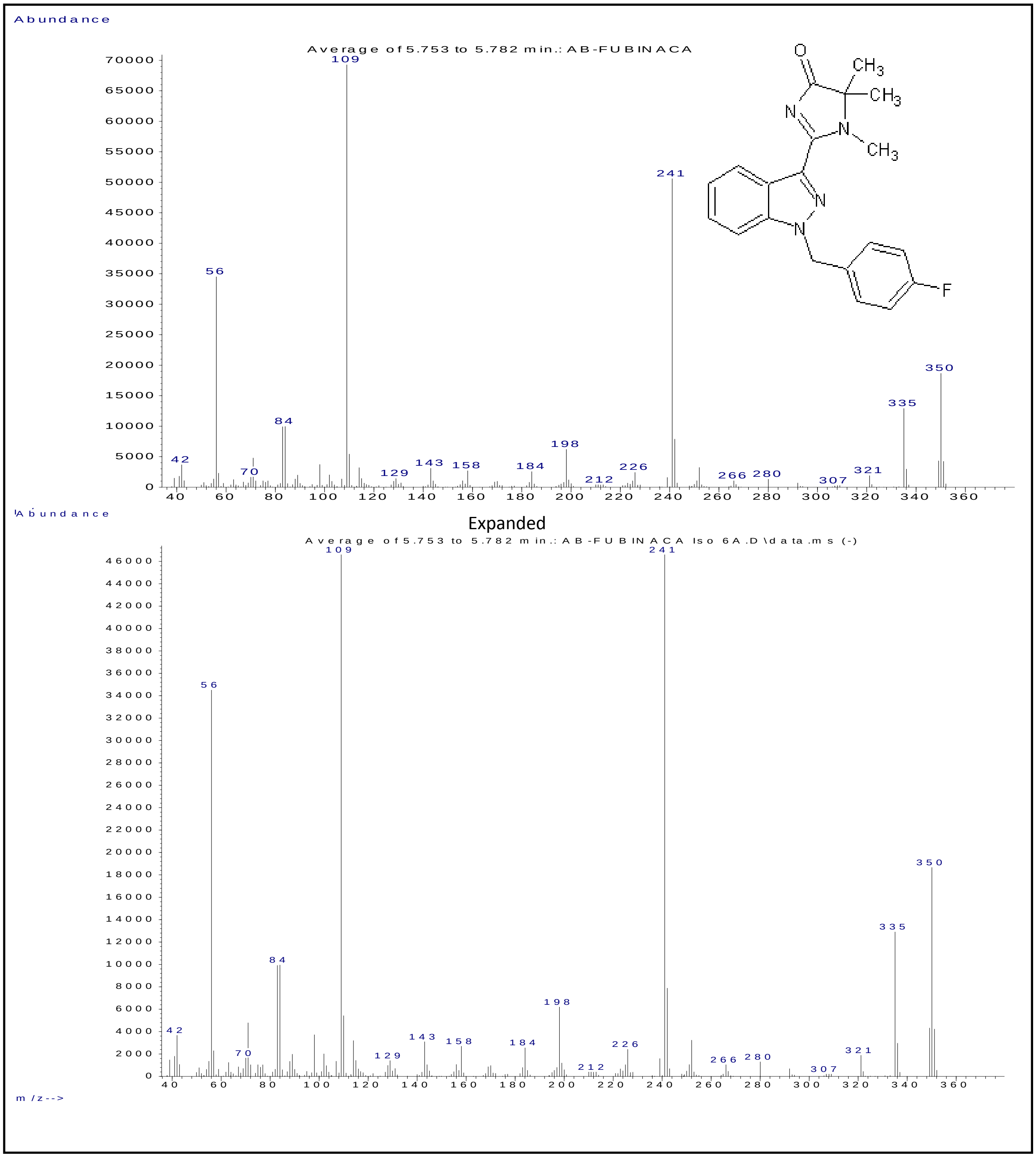
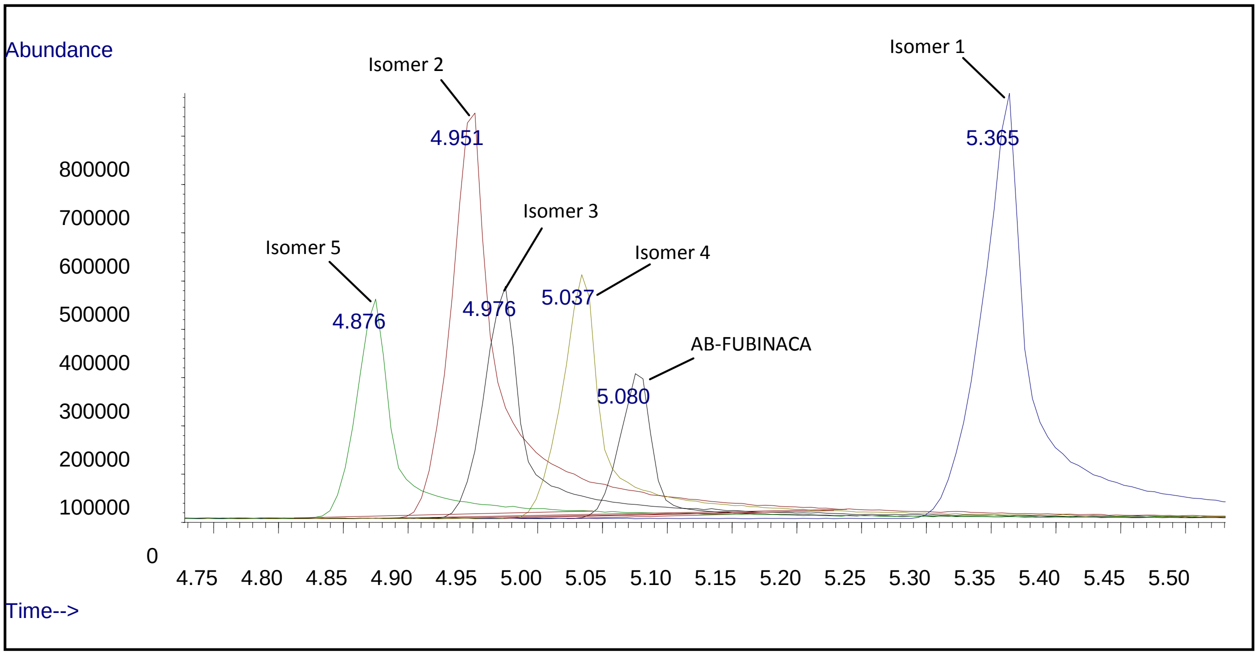


Figure 10. Overlay of AB-FUBINACA and Isomers 1-5 from Agilent 7890A GC/ 5975C MS



Gas chromatography / Mass spectrometry

Sample Preparation: Approximately 1 mg/mL in methanol

Instrument: Agilent 7890B GC/ 5977A MS

GC Parameter: **Column:** RXi-1MS

Carrier Gas: H

Oven Program: 120°C for 2 minutes, ramp 25°C/minute to 300°C and hold for 4 minutes.

Injection parameter: **Injector Temp:** 250°C

MS Parameters: **MSD transfer line:** 250°C

MS Source: 230°C

MS Quad: 150°C

Mass Scan Range: 35-550 amu

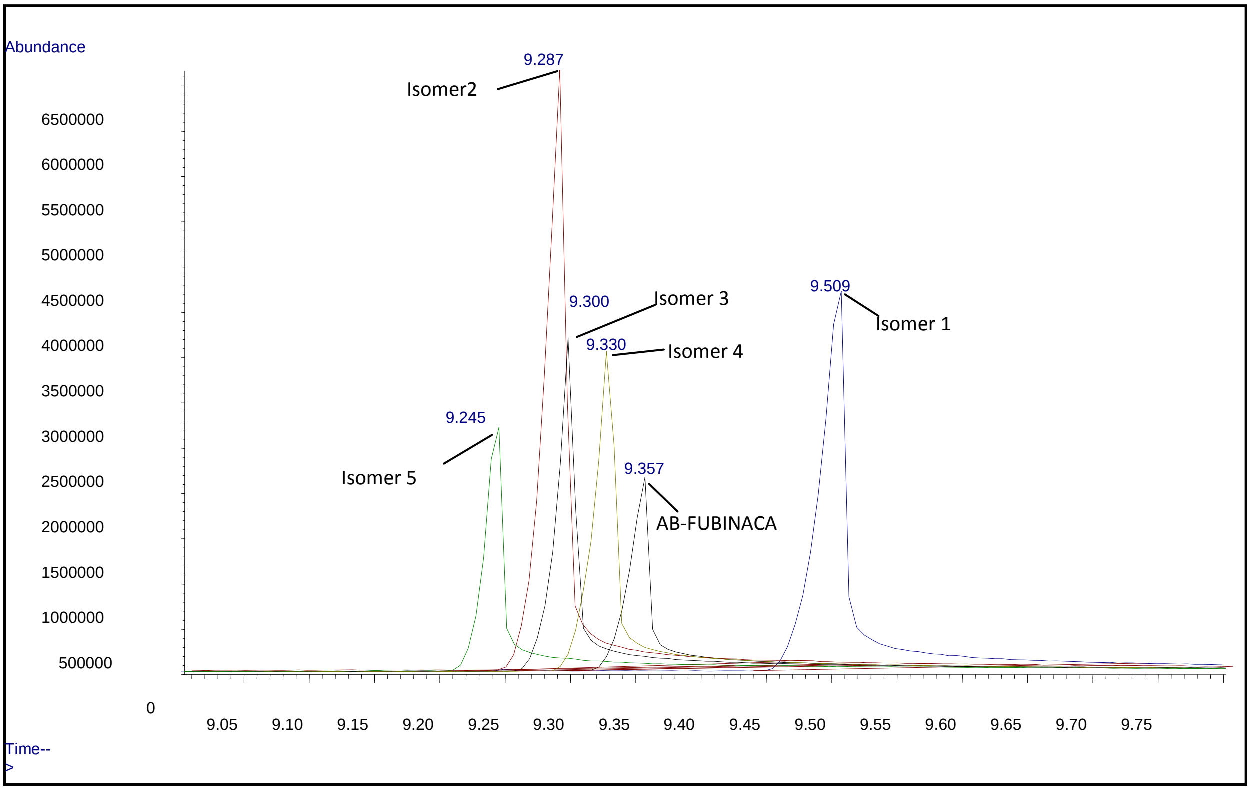
Threshold: 150

Tune File: atune.u

This GC/MS system was used to generate retention times and mass spectra using a different column and carrier gas. The mass spectra obtained using this instrument and method were very similar to those obtained above, and the retention times are listed below. The isomers eluted in the same order, as shown in Figure 11.

Compound	Retention Time	Relative Retention Time
AB-FUBINACA	9.357	1.000
Isomer 1	9.509	1.016
Isomer 2	9.287	.993
Isomer 3	9.300	.994
Isomer 4	9.330	.997
Isomer 5	9.245	.988
Isomer 6A	9.697	1.036

Figure 11. AB-FUBINACA and Isomers 1-5 overlay from Agilent 7890B GC/ 5977A MS



Gas Chromatography/ Infrared Spectroscopy (solid phase)

Instrument: Spectra Analysis DiscovIR/Agilent 7890A GC

GC Parameters:

Column: HP-5MS 30m× 250µm× .25µm

Carrier Gas: He

Split Mode: SPLIT

Split Ratio: 2:1

Oven Program: 240°C for 1 minute, ramp 30°C/ minute to 300°C and hold for 11 minutes

Injection Parameters:

Injection Volume: 1µL

Injection Port Temperature: 250^{o}C

IR Parameters:

Transfer Line: 290°C
Oven: 290°C
Restrictor: 290°C
Disk: -40°C
Resolution: 4 cm^{-1}

Figure 12. AB-FUBINACA GC/IR Data

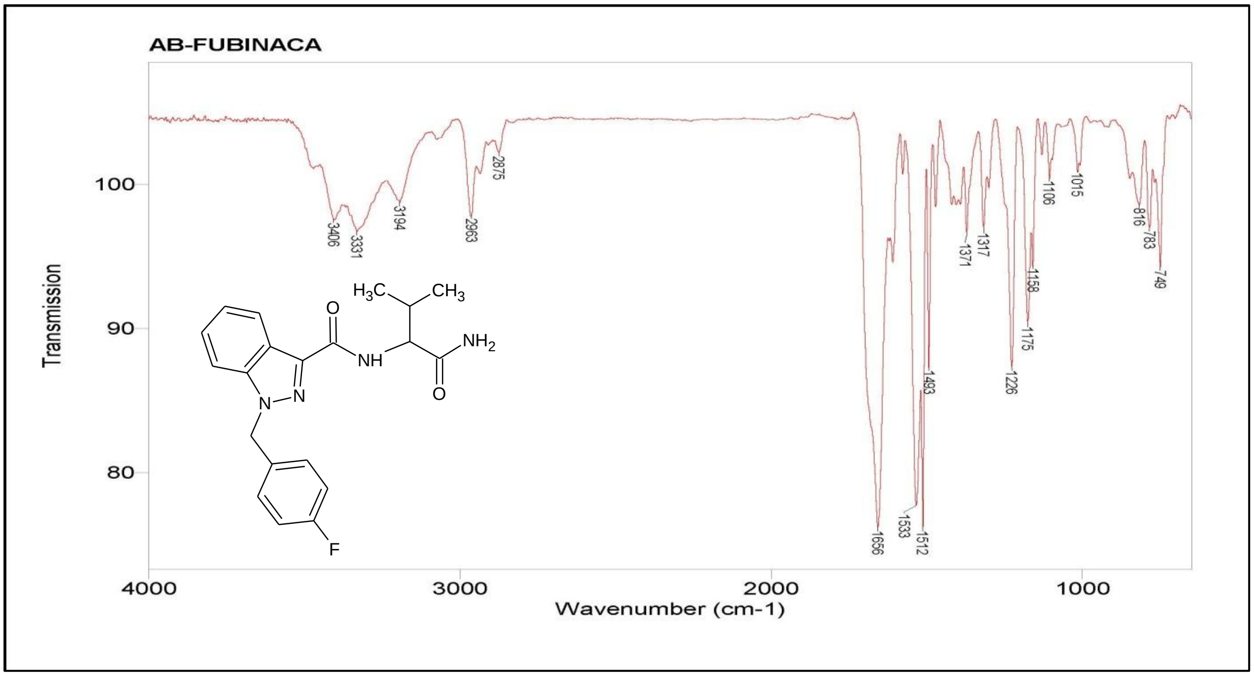


Figure 13. Isomer 1 GC/IR Data

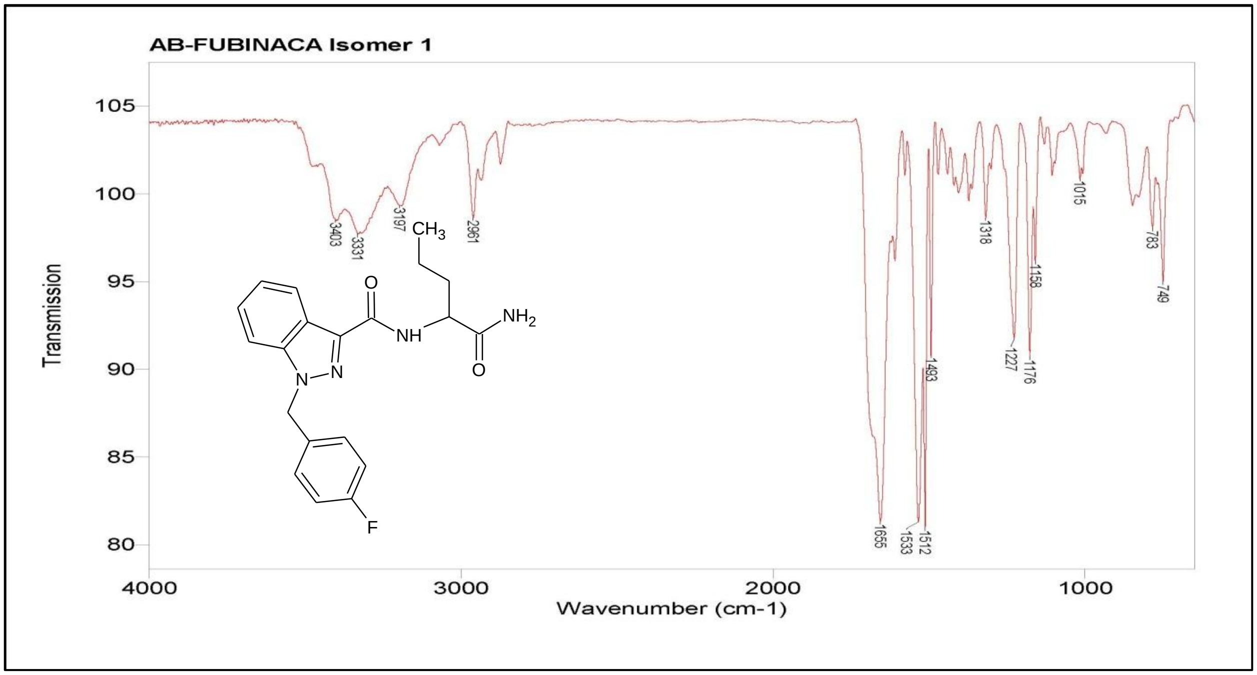


Figure 14. Isomer 2 GC/IR Data

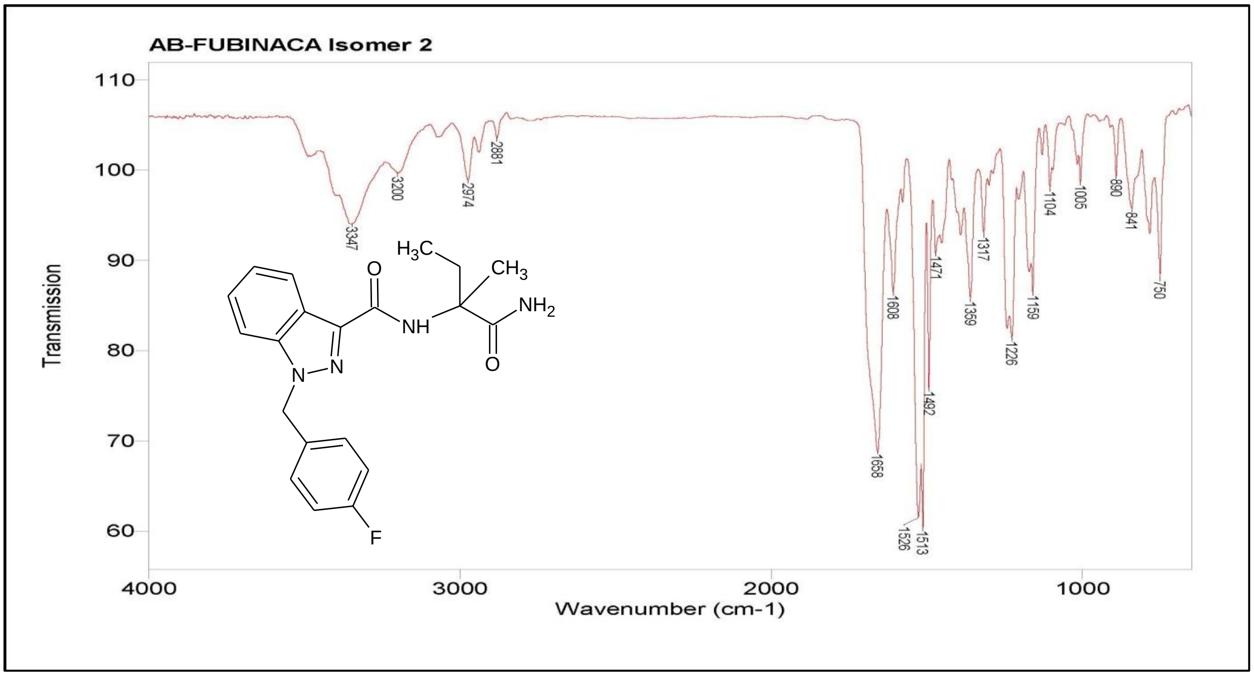


Figure 15. Isomer 3 GC/IR Data

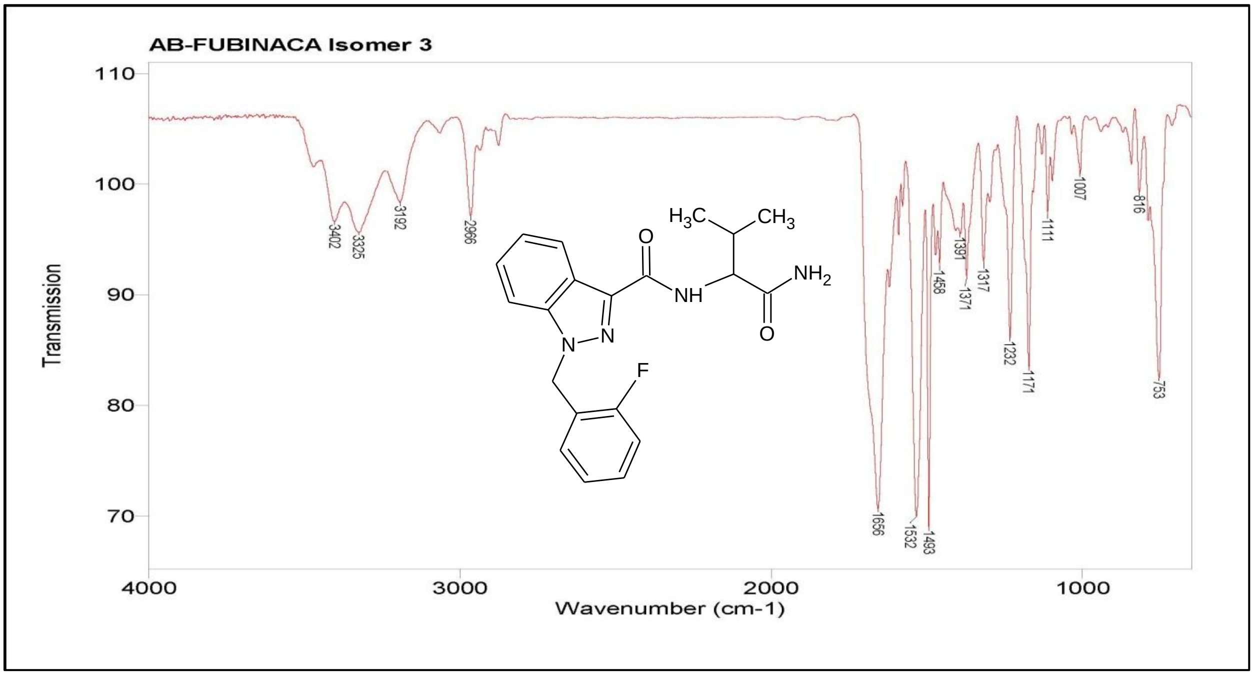


Figure 16. Isomer 4 GC/IR Data

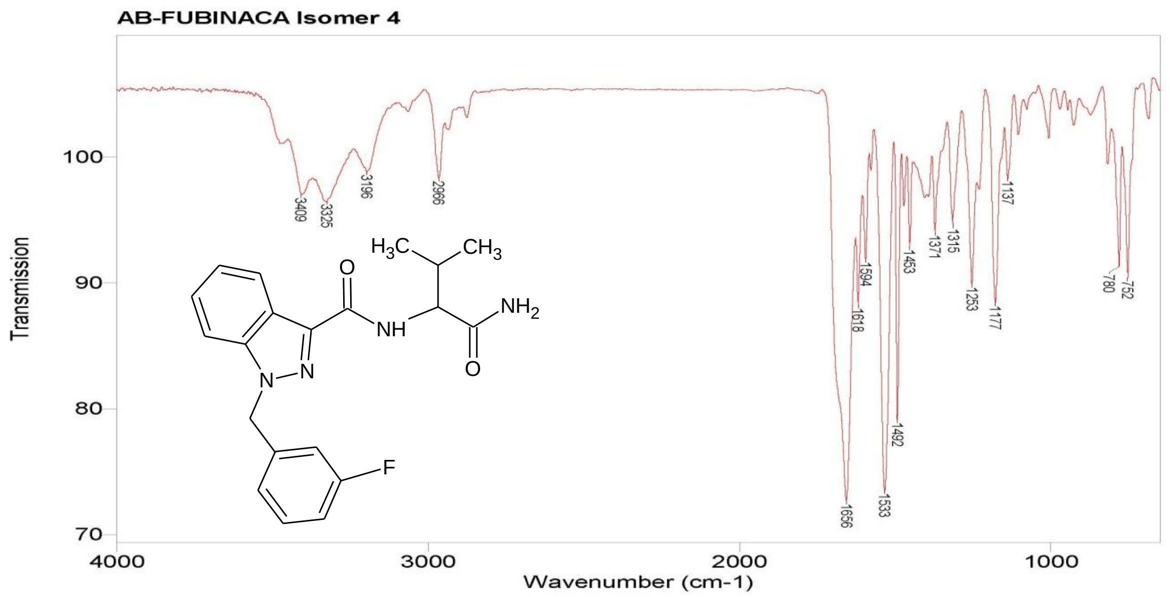


Figure 17. Isomer 5 GC/IR Data

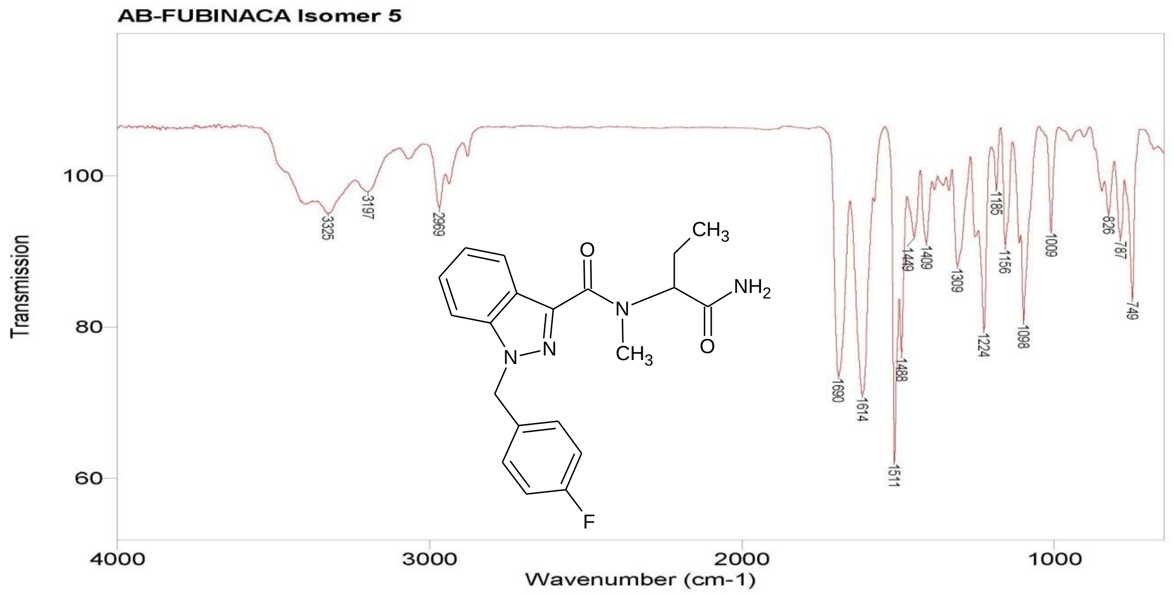


Figure 18. Isomer 6 Cyclized Degradant GC/IR Data

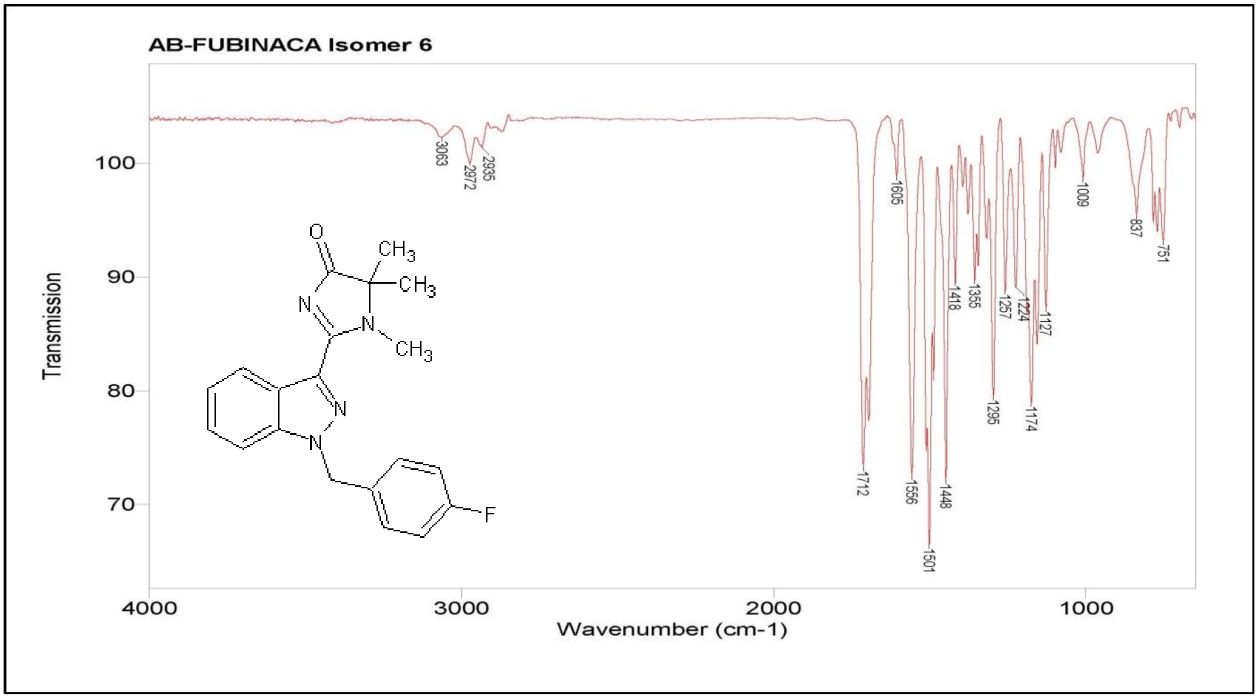
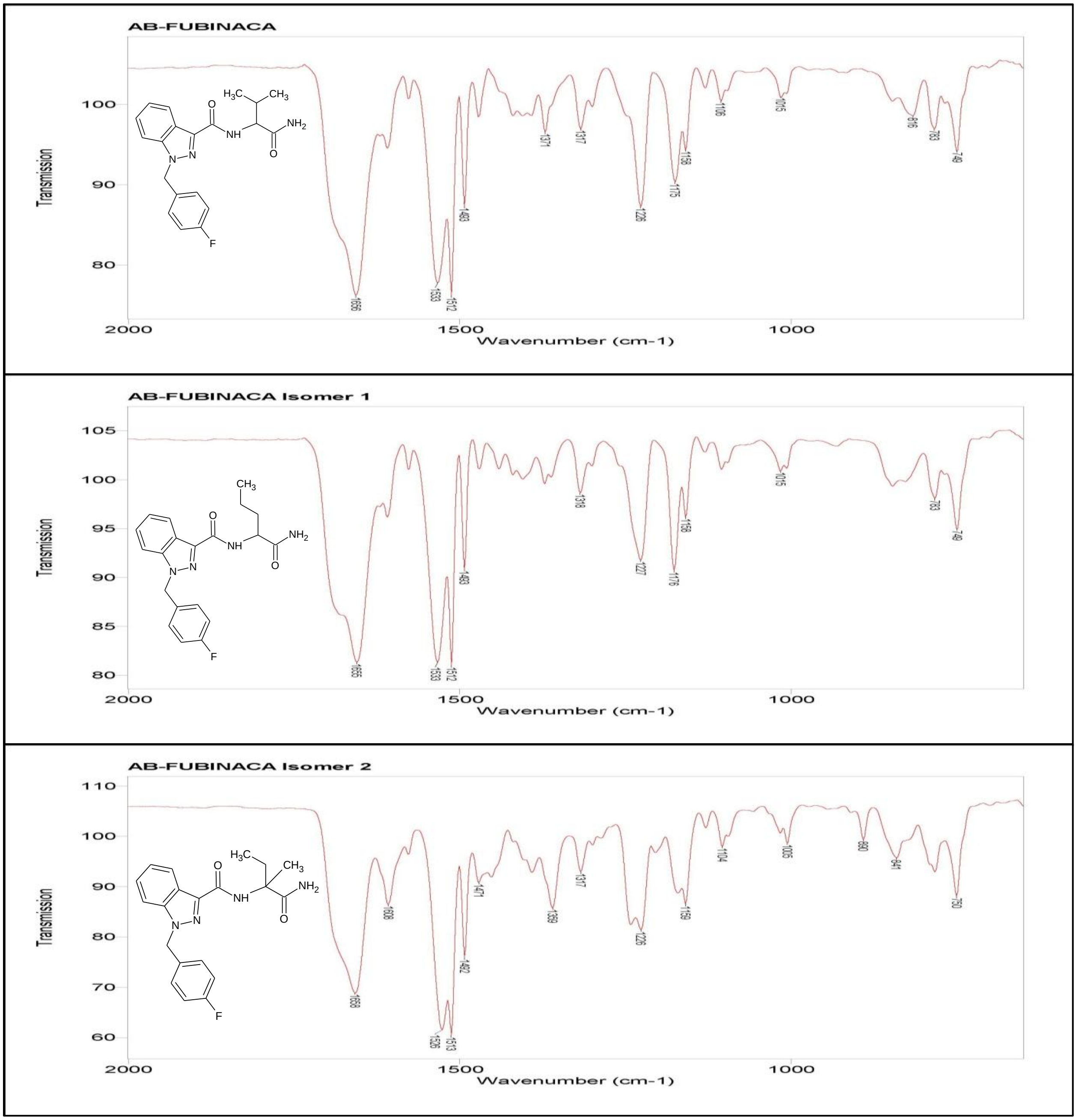
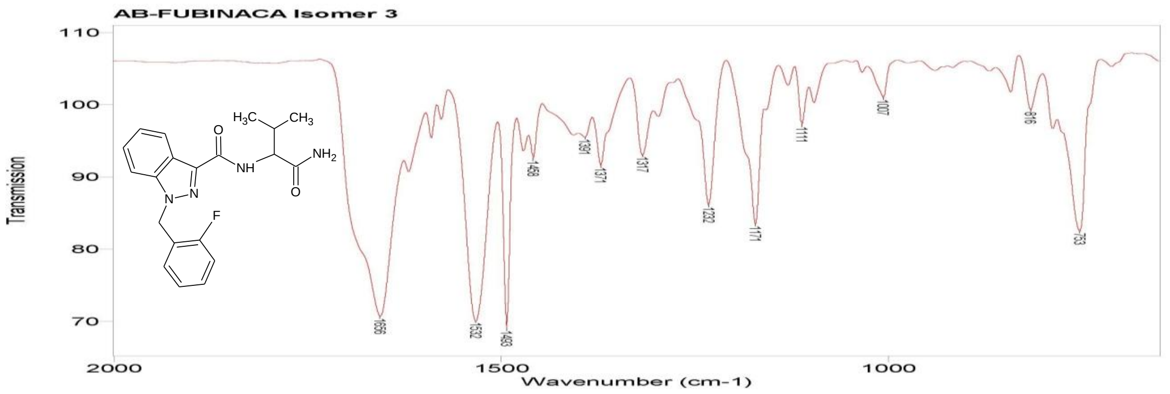
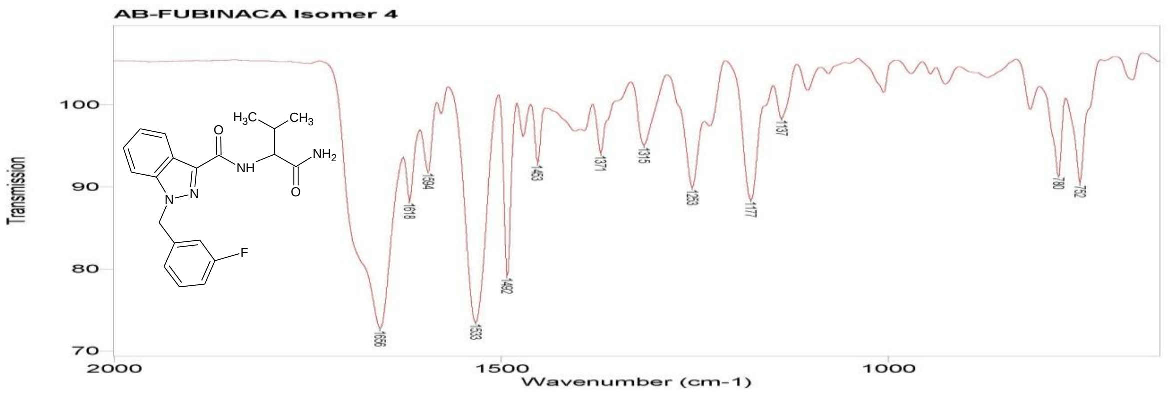
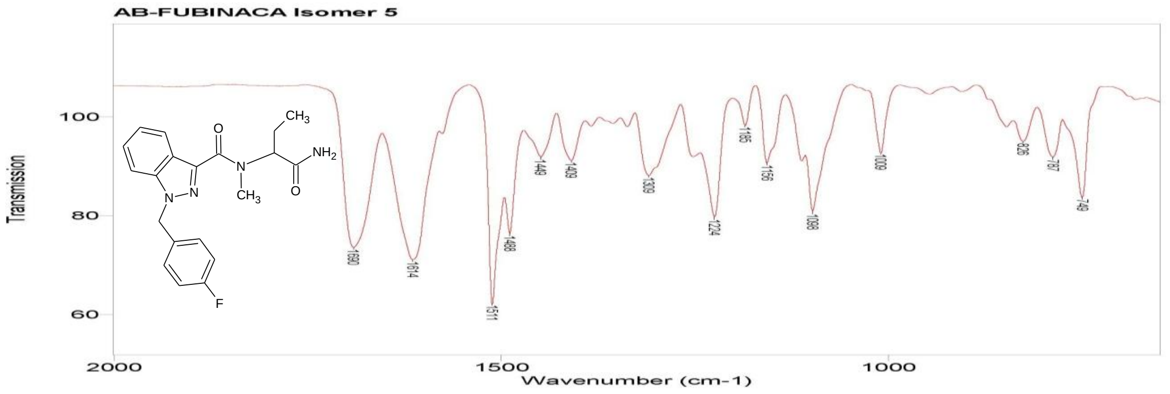


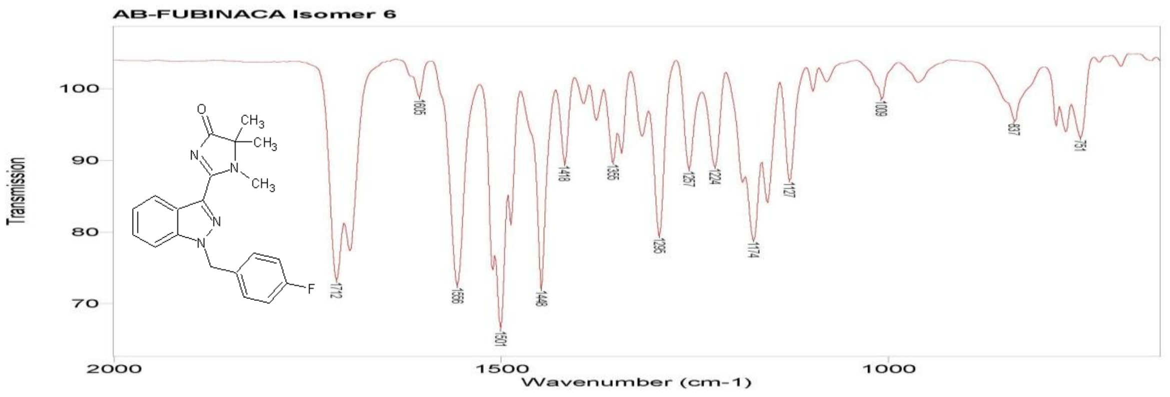
Figure 19. GC-IR Expanded Fingerprint Region Comparison




AB-FUBINACA Isomer 3


AB-FUBINACA Isomer 4


AB-FUBINACA Isomer 5


AB-FUBINACA Isomer 6

Part 2. External Links:

DEA SWGDRUG AB-FUBINACA Monograph

Forendex link to AB-FUBINACA

Wikipedia link to AB-FUBINACA